Citation:

Knopp RH, Retzlaff B, Fish B, Walden C, Wallick S, Anderson M, Aikawa K, Kahn SE. Effects of insulin resistance and obesity on lipoproteins and sensitivity to egg feedings. *Arterioscler Thromb Vasc Biol.* 2003 Aug 1; 23(8): 1,437-1,443.

PubMed ID: 12816878

Study Design:

Randomized Controlled Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To determine if insulin resistance with or without obesity influences LDL response to dietary cholesterol and saturated fat intake from eggs.

Inclusion Criteria:

None reported.

Exclusion Criteria:

- Cholesterol higher than 300mg per dL
- Triglycerides higher than 500mg per dL
- Diabetes
- Blood pressure higher than 150/100mmHg
- Renal, liver or unstable thyroid disease
- Coronary or peripheral vascular insufficiency
- Anemia
- Use of lipid-altering medications, beta-blockers, thiazides, corticosteroids, oral contraceptives and anti-convulsants
- Irregular menstrual periods, exceeding an interval of 24 to 32 days
- Ingesting more than two alcoholic drinks daily
- History of mental instability or mental illness
- Inability to comply with study requirements.

Description of Study Protocol:

Recruitment

Subjects were recruited using public advertising.

Design

- This study was a double-blinded, randomized, three-period crossover clinical trial
- It consisted of three one-month intervention periods with intervening one-month washout periods during which study subjects continued to follow the Step 1 diet
- Subjects were randomized to the order in which they would receive the egg preparation.

Dietary Intake/Dietary Assessment Methodology

- Three-day food records were kept at baseline and during each of the remaining study periods
- Dietary intake was reviewed by a registered dietitian (RD).

Blinding Used

Double-blinded study.

Intervention

The intervention consisted of daily ingestion of two or four eggs or an equivalent placebo. Daily portions of egg preparation were provided frozen, sufficient for one month, to be eaten ad libitum food choices at home within Step 1 guidelines.

- *Zero eggs (placebo):* 108g, 45kcal, 0g fat, 0mg cholesterol, consisted of Egg Beaters
- *Two eggs:* 108g, 171kcal, 10g fat, 425mg cholesterol; consisted of 34g egg yolks, 64g Egg Beaters and 10g water
- *Four eggs:* 108g, 298kcal, 20g fat, 850mg cholesterol; consisted of 68g egg yolks, 20g Egg Beaters and 20g water.

Statistical Analysis

- Sample size of 67 subjects per group was based on a significance level of 0.05, power of 0.80, and mean±SD difference of 9.3±17.6mg per dL LDL-C observed in a previous egg-feeding study in hyperlipidemic adults
- One-way ANOVA was used to test for statistically significant differences among the three groups, and the Games-Howell test, which adjusts for unequal sample sizes and group variances, was used to make multiple pairwise comparisons
- Baseline items with outliers or marginally normal distributions in one or more groups were also tested using non-parametric methods
- Chi-square tests were used to test for differences for categorical data
- Tests on baseline triglyceride and dietary cholesterol data were performed on log normalized values
- Significance testing was two-sided.

Data Collection Summary:

Timing of Measurements: Subjects make 10 visits over a 7-month period.

- *Visit one:* Study orientation, consent form signing, teaching of the Step 1 diet
- *Visit two:* Vital signs, body weight and height, lipoprotein profile and a fingerstick blood glucose measurement, along with a three-day food record review to ensure compliance with the Step 1 diet

- *Visit three:* Three-day food record review to ensure compliance with the Step 1 Diet
- *Visit four:* A frequently sampled, tolbutamide-modified intravenous-glucose tolerance test (FSIGT) and abdominal CT scan were performed
- *Visit five:* Vital signs, waist to hip ratio, diet evaluation, fasting glucose, insulin, lipoproteins and apoprotein B were measured, LDL size was measured and DNA was stored
- *Visits six to 10:* Vital signs, lipoprotein measurements, dietary intake evaluation were assessed at these visits, which occurred at the beginning and end of the four-week intervention periods. One month separated each of the intervention periods.

Dependent Variables

Lipoprotein analysis: Fasting total triglyceride, total cholesterol, LDL-C, HDL-C and plasma Apo B levels were analyzed using plasma.

Independent Variables

Egg consumption: The intervention consisted of daily ingestion of two or four eggs or an equivalent placebo, measured using three-day food records.

Control Variables

- *Weight status:* Determined using BMI calculated using measured height and weight
- *Insulin sensitivity:* Determined using fasting insulin.

Description of Actual Data Sample:

- *Initial N:* N=279
- *Attrition (final N):* N=197 (221 were randomized and 197 completed all feeding sequences)
  - 12 subjects who were obese and IS were excluded because of their small number
  - Nine subjects dropped out due to changes in their personal life
  - Six subjects dropped out due to failure to attend visits on time
  - Five subjects dropped out due to intolerance to the egg preparation.

Baseline Subject Characteristics

	Insulin Sensitive (IS) (N=65)	Insulin Resistant (N=75)	Obese Insulin Resistant (N=57)	ANOVA P-value
Age, year	49.4±9.3	55.0±11.5	54.1±9.1	0.0002
Female, percentage	66.2	57.3	57.9	NS
BMI, kg/m^2	23.2±2.3	24.5±1.9	31.5±3.8	0.000
White, percentage	93.9	92.1	93.1	NS
Insulin sensitivity, SI, 1x10^{-4} min^{-1} (µU per ml)	6.7±2.7	2.9±0.8	2.1±0.9	0.000
Cholesterol, mg per dL	186±37	210±31	206±38	0.000
Triglycerides, mg per dL	91±60	119±63	159±89	0.000

HDL, mg per dL	58±15	53±14	45±11	0.000
LDL, mg per dL	110±29	133±26	128±31	0.000
Apo B, mg per dL	85±21	102±20	105±25	0.000

- *Location:* United States.

Summary of Results:

- Mean fasting baseline LDL-cholesterol (LDL-C) levels were higher in IR and OIR subjects than in IS subjects (P<0.0001), and progressive triglyceride elevations and HDL-C decreases were seen across the three groups (see Table above in "Description of Actual Data Sample")
- Ingesting four eggs daily yielded significant LDL-C increases of 7.8%±13.7% (IS) and 3.3%±13.2% (IR) (both P<0.05) compared with 2.4%±12.6% for OIR (NS)
- HDL-C increases were 8.8%±10.4%, 5.2%±10.4% and 3.6%±9.4% in IS, IR and OIR, respectively (P<0.01).

Percent Lipoprotein Response to Egg Feeding (Mean Percentage Change ±SD)

	IS	IR	OIR	ANOVA P-value
Total Cholesterol				
Zero eggs	0.7±11.5	-3.0±8.1#	-1.6±8.3	0.067
Two eggs	2.2±9.1*	1.2 ±7.2	-1.6±10.0	0.044
Four eggs	6.2±8.7Ψ	3.2±9.9#	2.3±8.1*§	0.040
Triglyceride				
Zero eggs	9.9±48.1	2.6±45.5	8.1±45.7	0.626
Two eggs	-3.3±39.0	1.1±29,6	3.2±33.5	0.553
Four eggs	-5.5±36.9*	0.3±36.6	5.5±33.2	0.237
LDL-C				
Zero eggs	1.2±17.1	-2.2±14.3	2.1±19.4	0.285
Two eggs	3.3±13.8	1.5±10.8	-1.9±14.9	0.088
Four eggs	7.8±13.7Ψ	3.3±13.2*	2.4±12.6	0.051
HDL-C				
Zero eggs	1.5±10.7	-0.8±10.6	-2.0±11.8	0.188
Two eggs	5.5±10.3Ψ	2.2±8.8	-0.02±8.6£	0.004
Four eggs	8.8±10.4Ψ	5.2±10.4Ψ	3.6±9.4¥	0.014
Apo B				
Zero eggs	1.6±12.6	-1.9±10.4	-2.2±11.0	0.461
Two eggs	3.4±10.9	2.1±7.6	-1.6±9.4	0.230
Four eggs	9.5±12.7#	4.2±11.3	2.4±9.1	0.107

Significantly different from before feeding to after feeding: *P<0.05, #P<0.01, ΨP<0.001.

OIR vs. IS: §P<0.05, ¥P<0.02, £P<0.01.

Author Conclusion:

- Insulin resistance with and without obesity is associated with elevated LDL-C, as well as elevated triglycerides and low HDL-C
- The elevated LDL-C cannot be explained by dietary sensitivity, because the LDL-C rise with egg feeding is less in IR persons regardless of obesity status.

Reviewer Comments:

None.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes

2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	No
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	No
4.4.	Were reasons for withdrawals similar across groups?	???
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes

5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes

7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	No
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	Yes
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	No
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes